THE LARVA OF CTENOPHORA ANGUSTIPENNIS LOEW.

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I. INTRODUCTION.

The great majority of the Dipterous insects are unfortunately known only in the adult state. This lack of knowledge in regard to the larval and pupal conditions of many forms leaves a gap which cannot be filled for some time. This gap, as pointed out by Kellogg, is especially noticeable in the case of the lower forms in their immature stages, which have hardly been studied at all. This lack is particularly serious inasmuch as these are the more generalized forms, and represent the ancestral types from which the highly specialized Tipulids have been evolved. It is to these lower forms that we must look for information regarding the genesis of the group.

The larva of Ctenophora angustipennis is peculiarly interesting, and as the main anatomical features can readily be worked out, it offers a most suitable subject for study in elementary entomology. The chief structural details can even be made out in observing the live specimen and the larvæ can usually be secured in sufficient abundance to furnish plenty of material.

II. Habitat and Mode of Life.

Ctenophora larvæ are somewhat gregarious in habit and are usually found massed in runways, as it were, in much decayed cottonwood or alder logs. They are rarely found in other logs and never in such great numbers. Where a single specimen is found a half hundred or more will commonly be found in the same log, though sometimes only a few will be secured. As the larvæ require moisture, they are usually found in the soft, punky wood along the lake shores. The numerous specimens used in this study were all secured from the small strip of the Lake Washington shore on the university campus. The larva probably obtains its food supply from the bacterial life found in this spongy wood; as when many of them are kept for some time in the same material they gradually absorb the plentiful adipose tissue, which apparently furnishes a reserve food supply. Numerous protozoan parasites are frequently found in between the cells of the alimentary canal, especially in the proventricular cæca.

III. Description of the Larva.

The Ctenophora larva (Fig. 1) is cylindrical, tapering somewhat towards the hinder end, and is bluntly rounded in front, especially when the head is much retracted. The larva is from three-quarters of an inch to a trifle over an inch in length. There are no external appendages or protuberances, but the animal is found covered with numerous fine hairs, extending backwards. When the larva is held to the light, these cause it to appear yellow on the edges. The larva moves quite freely by vermiform movements, and these are possibly facilitated by the presence of the hairs. The body proper consists of eleven segments, which, with the exception of the first and last, are not clearly marked off. Most of these segments are subdivided into annuli, but as the number of annuli varies in different specimens, and even in the dorsal and ventral portions of the same individual, their morphological value must be slight. The prothoracic segment, the one just posterior to the head, appears at first sight double, as it is divided transversely by a distinctly-marked fold. This fold, however, is merely the result of the very frequent retractions of the head, which may be completely withdrawn within the first segment.

Indeed, it is generally so withdrawn, except when the animal is moving or eating. The head is thus surrounded by a fold of the body wall which greatly facilitates the retraction and protrusion of it. The top of the head (Fig. 2) is defended by a strong chitinous shield. The occipital region of this, as is the usual case in the retractile head of the Dipterous larvæ, is imperfectly chitinized and is posteriorly excavated by two deep notches. The antennæ (Fig. 3) are single jointed, projecting a little from the side of the head. No eyes are visible.

The extreme posterior segment (Figs. 4, 5 and 6) is modified as usual. The anus opens at the apex of this segment. Just above the anus and on each side of the median line are the two large oval stigmata (Fig. 7). The elliptical central core or plug looks coal black, while the surrounding ring of irregular chitinous threads is of a deep brown. The stigmata are of the primitive or generalized type, and are, therefore, without lips. The aperture can be closed by bringing down the surrounding lobes. The spiracles are surrounded by six backward projecting flexible lobes, four of these are in a line above the spiracles, while the others are just below and on each side of the anal opening. When these are contracted they serve to protect the stigmata, and are strengthened by the presence of small chitinous patches on the posterior tips.

As the larval skin is quite transparent, the main body systems can easily be seen through the skin. If the fat-bodies are well-developed, as they are just before pupation, they completely envelop the alimentary canal and therefore the larva appears white and opaque. When the live animal is observed the heart can be seen as a delicate pulsating tubule, which lies in the median dorsal line, extending from the second to the extreme segment. The much darker alimentary canal shows on either side of the heart, while the large lobulated cæcum extends two-thirds of the way up the left side. The superficial tracheal system, which consists of the two main lateral trunks with their cross-connections, stands out as slender, shining white strands. On the ventral surface, the ventral ganglia, with their lateral branches, show very clearly. It is this readiness with which the main anatomical features can be made out that renders Ctenophora larvæ such remarkably fine subjects for students in entomology.

The mouthparts are very complex, and their exact homologies cannot be accurately determined save by a very extensive embryological study. The short single-jointed antennæ arise from the small lobe of the plate covering the top of the head. The antennæ bear at their extremity two groups of sensory papillæ. The mentum is triangular, with three serrations on each side and a larger apical tooth. The mandibles (Fig. 8) are strong and heavy. On the inner side of each mandible there is attached the serrated mandibular lacinia, which is evidently of great importance, as it is so very well developed. The structure of the maxillæ (Fig. 9) is extremely complicated and no definite homologies can be made out. The lacinia, a row of fine projections, lies next the mentum. On the outer side a very short palpus shows, and is carried on the short, curved palpiger. Back of this extends the head sclerites.

The entire body of the larva is covered with fine, close-set, simple hairs, pointing backwards. These are very much thicker on the anterior part of the body, and gradually decrease in number toward the posterior. This fact of distribution would tend to show that these hairs are somewhat sensory in function. Besides these there are larger hairs (Fig. 10), collected here and there in groups of from two to six and probably sensory in function. These large hairs are hollow with a central pore canal and are, in common with the shorter ones, somewhat useful in locomotion.

The skin is very inelastic and tough, due to the deposit of the thick chitinous layer. The skin consists primarily of an irregularly curved layer of columnar epithelial cells, the hypodermis. This layer secretes the chitin, which is laid down in irregularly waved laminæ.

The muscle system is very complex and there are a great number of muscle bands. There is a wide band of longitudinal muscles along the side of the dorsal and ventral median line of the body. Some of the fibers reach from the exterior to the posterior border of each segment, but other fibers reach from the middle to either end, and still others reach from the middle of one segment to the middle of another. There is also an inner set of lateral transverse muscles. Each muscle is a bundle of long fibers, each of which is enclosed in an outer elastic membrane, the sarcolemma. Each fiber is in turn made up of several fibrillæ. The muscle fibers of the insect present a beautiful striated appearance, which is due to the alternate light and dark bands of substance. In life the muscles are colorless and transparent. They are so soft that they are of a gelatinous consistency.

IV. Methods of Killing and Staining.

Hot Gilson's fluid was found to be the best reagent for killing the larvæ. With this the animals were killed very quickly and without contraction, while a slower reagent caused much distortion of the tissues. It was found impossible to make paraffin sections on account of the tearing of the rest of the section while cutting through the thick chitinous wall. Excellent preparations may be made by the celloidin method, although even here there is danger of tearing the inner delicate tissues. The best stain for use in making out the general differentiation of the tissues was found to be Bohmer's hæmatotoxylin. This stain was also used to good effect in working out the finer histological details, such as the cell structure of cæcum, the finer details of the muscle structures, and the details of the nervous system. Iron hæmatoxylin was found an especially good stain for working out the cell structure of the salivary gland and the proventricular cæca. For the differentiation of the muscle plexus of the cæcum, Congo-red was the most satisfactory stain. In preparing whole mounts of the cæcum the best fixation was obtained by slitting the animal open for a short distance along the side and at once immersing it in a weak Flemming's solution. The best mounts of the mouth parts were made by removing the organs and cleaning them in strong carbol-xylol with a little safranin in the clearing solution.

V. The Digestive System.

The digestive system of Ctenophora is very like that of Holorusia, the allied giant Tipulid, as described by Kellogg. (Psyche, June, 1901.) The exceedingly large diverticulum is characteristic of the vegetable feeding larvæ of Tipula and Ctenophora. The alimentary canal extends as a straight tube from the anterior to the posterior extremity of the body, and is nearly wholly enclosed in the coiled perforated sheets of adipose tissue. (Fig. 11.) The tube consists first of the long slender œsophagus, which opens into the hypopharynx. At about the middle of this, the œsophagus is embraced by the circumœsophageal commissures and the brain lobes. At its posterior end, the œsophagus suddenly dilates and passes into the proventriculus, whose diameter is about ten times as great as that of the œsophagus.

The finer structure of the œsophagus, as seen in a cross-section, differs in the anterior and posterior part of the tube. (Figs. 12 and 13.) The outer œsophageal layer is composed of a band of circular muscles, beautifully striated and showing large oval nuclei. These nuclei extend over several striations. Within this is a doubtful layer composed of a few strands of longitudinal muscles, which lie in the cavities formed by the invagination of the œsophageal epithelium. At places this epithelium is contiguous with the circular muscles and show no trace of any longitudinal muscles. Within this muscle layer is the much convoluted epithelial layer. This is composed of a single layer of columnar cells and is thrown into numerous, deep, irregular, longitudinal folds, which at times cause the lumen to be almost closed. The inner margin of the epithelial layer, unlike that of the epithelium of the cæca, forms a straight line. This layer secretes the heavy, inner chitinous layer, depositing the chitin in irregular laminæ. In the anterior portion of the tube the lumen is nearly filled by the long, stiff hairs which project into the cavity. (Fig. 14.) These hairs are entirely lacking in the posterior portion, which is also distinguished by having no signs of the longitudinal muscle strands.

The proventriculus comprises the adjoining abruptly dilated portion. At the point of juncture the œsophagus is surrounded by the fine sphincter muscle, so that the entrance to the œsophagus may be completely closed and so keep food from passing back up the œsophagus. (Fig. 15.) There is also a large œsophageal invagination. This has the same cell structure as the posterior portion of the œsophagus, and extends for about the length of the proventriculus, when the line separating the ventriculus and the proventriculus is taken to be the beginning of the ventricular cæca. The wall of the proventriculus is composed of a single layer of secreting epithelium and is surrounded by a thin muscular membrane. This membrane is composed of a layer of circular muscles and an incomplete layer of longitudinal muscle fibers. The epithelial layer shows no signs of great activity as the inner cell margin is straight and no discharged globules are present.

There is no sharp line of division between the proventriculus and the vertriculus. The ventriculus proper bears at its anterior end four elongated pouches, the ventricular cæca. These are not alike in structure, two of them being nearly twice as long as the other two. They differ in this respect from the gastric cæca of Holorusia, where all four are of the same size. The longer cæca extend along the ventriculus for about one-fifth of its entire length. The transition of the epithelium of the ventriculus to that of the cæcum is very sudden. (Fig. 16.) The epithelium exhibits a very different appearance according to the degree of secreting activity. The cells of the cæca are evidently very active in secretion and exhibit many granular protrusions. In the longer cæca there is but a single layer of cells, while in the shorter cæca there are two incomplete layers. (Figs. 17 and 18.) The cells of the shorter cæca are evidently much more active and show many narrow-necked protrusions. These protrusions increase in size till the cell projects into the lumen; the connection between the cell and the protrusions constricts, and there results a separation of the spherical globule. After the globule becomes free in the lumen it loses its definite outline. (Fig. 19.) The globule is finely granular, but nowhere contains any sign of a nucleus, such as described by Needham (Zoological Bulletin, 1897) in the digestive epithelium of the dragon-fly nymphs. The discharged portion of the cell represents but a small part of the whole and does not contain any trophic center. The secretion here differs from that of the larva of Ptychoptera as described by Gehucten (quoted by Packard, pp. 326-329) for the globule is constricted off and does not burst through the cell membrane. The process closely resembles that of Collembola, although there are no such marked changes in cell alveolation. (Folsom and Welles, in the Univ. of Illinois Bulletin, 1906.) Many protozoan parasites are found in the cæca, being often wedged in, as it were, between the cells. (Fig. 20.)

Near the posterior termination of the ventriculus are four very small protruding pockets, the gastric cæca. (Fig. 21.) These pockets have not been described as occurring in the nearly allied form, Holorusia. The epithelium here is composed of two layers of cells and is very much convoluted. The cells are evidently most active and show many protrusions and discharged globules.

The termination of the ventriculus is marked by a pale transverse line and the four coiled Malpighian tubules pass off at this point. Each tube passes forward to the base of the ventricular cæca and then turns backwards. In a cross-section the tubules show a ring composed of from two to six large polygonal cells, which have very conspicuous nuclei. The excretory canal is clearly marked. (Fig. 22.)

Back of the ventriculus lies the small intestine, which is of smaller caliber than the ventriculus. The diameter of the small intestine is only about one-fourth of that of the ventriculus. This opens into the fifth division of the alimentary canal, the large intestine. The large intestine is distinguished by the very large intestinal cæcum or diverticulum, which is one of the distinguishing features of the herbivorous Tipulidæ. This cæcum lies along the ventriculus and extends as far as the proventriculus. A somewhat similar surface is present in Holorusia, but it is only about one-third as large. On a surface view the cæcum appears flabby, this being due to the pouch-like effect produced by the transverse and longitudinal muscles. The walls of the diverticulum are very thin. The wall is composed of two layers, an outer one made up of annular muscles and an inner one composed of very large cells. (Fig. 23.) (Fig. 24.) The muscles of the diverticulum form a most peculiar network or muscle plexus, as it might be called, which in its branching and anastomosing bears a superficial resemblance to a nerve plexus. (Fig. 25.) There are eleven or more large bands of striated muscles which extend around the diverticulum, and these are connected by numerous finer cross-branches, which are also striated. (Figs. 26 and 27.) In a few places the muscles seem to radiate from a central mass, but in general the ladder-like appearance is very marked. The striations run across both the large muscles and the finer connecting bands, so that the striations at the junctures meet at right angles. (Fig. 28.) There are many muscle nuclei, which, on a side view, seem to stand out from the fiber itself and to be surrounded by a transparent wall. By means of this peculiar muscle plexus, the diverticulum can be contracted in all directions at once. The gross structure of the plexus may best be demonstrated by mounting the entire wall of the cæcum after first removing the inner cell layer by means of a very fine brush. The cellular membrane is also remarkable. The individual cells are very large and have very conspicuous nuclei. (Fig. 29.) There is a close resemblance in structure between the cells of the intestinal diverticulum and those of the Malpighian tubules. The cells are but loosely approximated to the muscle walls and in places the cells are separated by distinct canals. (Fig. 30.) The nuclei are very large, with deeply staining chromatic filaments. This large diverticulum, the presence of which characterizes the herbivorous Tipulids, is excretory in function, though it may also act as a sort of food reservoir. The similarity of cell structure with that of the urinary tubules would seem to point to this conclusion, but besides this there is the additional evidence of chemical tests. There is a strong uric reaction to the murexid test, which would seem to show conclusively that the diverticulum, as well as the Malpighian tubules, is excretory in function.

The large intestine dilates gradually till it forms the rectum. In cross-sections the structure appears very similar to that of the œsophagus. The epithelial surface is thrown up into numerous irregular longitudinal folds. The cells are uniform in size, and show no signs of any activity in secretion. Within this epithelial layer there is a layer of chitin, which is thrown into irregular folds and laminæ. Outside of the epithelial layer there is a series of striated annular muscles, which extends outwards for a distance equal to about one-fourth of the central cavity. (Fig. 31.) The structure of the rectum proper is very much like that of the colon, and the transition is imperceptible, but here the folds of the columnar epithelium extend much farther into the lumen and the chitinous layer is so thick that it almost obliterates the central cavity. The muscular ring is much thicker, extending out for a distance equal to the diameter of the lumen. (Fig. 32.) The heavy muscular walls serve to retain the food in the absorptive portions of the digestive tract till all possible nutriment has been extracted.

There is a much coiled salivary gland lying on each side of the œsophagus. (Fig. 33.) Each consists of a greatly coiled tubule, with a slender collecting duct extending from the anterior portion. The two collecting ducts unite to form a common duct which lies just beneath the œsophagus and opens at the base of the hypopharynx. The glands are hollow, the walls being only one cell thick. The cell wall consists merely of an epithelial layer with its intima and basement membrane. As seen in a surface view the cells are very large and polygonal in shape. (Fig. 34.) They possess very large nuclei in which a distinct chromatic filament can easily be made out by proper staining. The breaking down of the salivary gland is accompanied apparently by simple cell degeneration, the "selbständige" degeneration of Karawaiew, and therefore without the recurrence of phagocytosis. The cell nuclei are at first regularly circular and sharply marked out by a nuclear membrane, but later on the nucleus loses this membrane. (Fig. 35.) The histolysis here follows closely the course described by Kellogg in regard to Holorusia. (Am. Nat., 1901.) Of course in the case of such a generalized larva as that of Ctenophora the breakdown of the larval organs would be accomplished with less change of structure than in the more specialized forms, which have been largely the forms studied in this connection, and so there would be less reason for the occurrence of phagocytosis.

VI. The Tracheal System.

There are two main lateral tracheæ, one passing along each side of the medio-dorsal line. These main divisions of the respiratory system are seen very clearly when looking at the live larvæ, and appear as two glistening bands when the animal is expanded, but having a sinuous course when the animal is contracted. These two tracheæ are connected by transverse and anastomosing branches, one main connecting branch in each segment. These branches, as well as the lateral tubes, give off numerous side branches. As these commissures are connected with the alimentary canal they are very slack, especially those near the middle of the body. During the vermiform movements of the larva, there is a great deal of sliding of the body wall and the alimentary canal, and the commissures must be able to stand the strain.

From these two main lateral branches ramifications pass off into every part of the body. (Fig. 37.) These branches pass among the different organs of the body and seem to serve somewhat as strands to hold them in place. These branches have very minute ramifications, which become so attenuated that they pass among the fibers of the muscles. Each of the main tracheæ at the anterior extremity breaks up into a number of fine fibers, most of which are connected with the brain. In the last segment, just above the hinder part of the heart, there arises a number of small branches which are connected with the stigmata.

The lining membrane of the tracheæ consists of a layer of polygonal cells fitting closely together as a pavement epithelium. The chitinous wall or intima is thickened at intervals to form thread-like ridges, the tænidia. These tænidia serve to keep the tracheæ open without affecting their flexibility.

There is but one pair of spiracles so that the larva is metapneustic, as is the case with nearly all the Tipulidæ. The prominent oval spiracles are inserted at the apex of the last segment, and turn backwards and upwards. The structure of the spiracles is very similar to that of the larvæ of Dicranota and Phalacrocera, as described by Miall and Shelford. (Fig. 38.) The central portion of the spiracle consists of an inner cone or plug, which is surrounded by a thick and solid chitinous wall. Outside of this is a chamber with a colorless chitinous wall, the vestibule. The cavity of the vestibule is crossed by many radiating fibers, which are irregularly branched and start from the outer wall. Some of the fibers connect with the inner cone, but many do not reach it at all. The stigma forms the outer end of an air chamber whose inner surface is lined with a zone of large cells. This region is evidently of great importance in the respiratory mechanism of the larva, as there are great masses of blood corpuscles lying about the spiracle. There is a circular muscle with which to draw in the spiracle and help bring the fleshy protuberances about it. The lateral tracheæ lost their tænidia just before they join the floor of the vestibule. The minute tracheal ramifications which extend out from the sides of the vestibule are not brought into any direct contact with the main vessel, but serve rather to aerate the numerous blood corpuscles. The large tracheæ lead out from the spiracle and give off numerous branches to the body wall and viscera. There is connected with the stigmatic region a very minute nerve plexus, which helps to show the great importance of this region in respiration and circulation.

VII. The Circulatory System.

The dorsal vessel or heart is a slender delicate membraneous tubule which lies along the medio-dorsal line, and extends from the brain to the last segment. In the live animal this may be seen to pulsate. The heart is cut off from the body cavity by the usual diaphragm. This diaphragm extends outward from the heart and, with the dorsal wall of the body, forms a pericardial chamber. The diaphragm is formed largely of paired fin-like muscles, the alary muscles, which extend past the lateral tracheæ and connect with the body wall. There are apparently no ostia, but at intervals the wall is thickened and the heart is partially divided into a series of chambers. This lack of ostia is not an aberrant condition, as there are no ostia present in the very young larva of Musca (Kolbe). The heart is attached to the body wall by the very minute suspensory muscles. These are attached to the upper surface of the heart and radiate till they come in contact with the body wall.

In a cross-section the heart is somewhat lozenge-shaped. (Fig. 39.) There are three distinct layers which compose the heart, as may be seen in a cross-section. There is (1) a very fine, transparent, and structureless intima, which is not marked except under a very high magnification, (2) a central layer of circular muscles, which effect the contraction of the heart, and (3) there is an extremely thin enveloping layer, the endocardium.

Along the heart on the basal side and along the alary muscles occur the so-called pericardial cells, which from their position would seem to have a close relation to the circulation of the blood. Some œnocytes are at times found collected along the body wall in the posterior region, as described by Bengtsonn. According to Kowalevsky the function of the pericardial cells is to remove the foreign or injurious matter mingled with the blood. The pericardial cells themselves are elliptical in shape and with no easily distinguished cell wall. They often have two nuclei lying side by side in the cytoplasm and sometimes as many as four without any sign of a separating cell wall. The cell-nuclei stain very heavily, but show no distinct chromatic filaments. The pericardial cells are congregated about the sides of the heart and lie along the alary muscles for some distance from the heart.

The blood proper is a thin whitish fluid which contains the very pale oval corpuscles. These corpuscles have a rounded nucleus and are covered with fat globules. The fresh blood has a slightly alkaline reaction. These leucocytes are seen in great numbers at the posterior end of the heart, just in front of the stigmata. At the stigmata the blood is passed over the tracheal ramifications, so that it tends to traverse the normal condition in insects in which the air is always brought to the blood; while here the blood seems to be brought to the air.

VIII. The Fat-bodies.

The alimentary canal is nearly enclosed in the thin sheet of adipose tissue, which is perforated by many small holes. This tissue fills in the space between the other organs and so occupies a large part of the body cavity. The cells are regularly polygonal in shape, and possess a central oval nucleus. (Figs. 40 and 41.) The fat-bodies are probably reserve food material to be used during the rapid pupal metamorphosis, as the sheets of adipose tissue are very large and conspicuous in larvæ which are about to pupate. When the larvæ have been kept for some time without any adequate food supply the fat-bodies are gradually absorbed.

IX. The Imaginal Buds.

The imaginal buds are small whitish bud-like bodies which lie between the muscles and the body wall of the thoracic segments. There are two pairs of these imaginal buds in each segment. There are several present on the ventral surface of the live animal when studied with a hand lens. The ventral invaginations give rise to the legs, while the dorsal invaginations develop into the pupal respiratory organ, the wing, and the halterer.

The imaginal buds or histoblasts, which Kellogg suggests as a better name for these structures, are composed of an invaginated portion of the hypodermis which has become folded and in which there has been a special increase of cells. (Fig. 42.) During this modification, the outer portion of these cells is separated from the rest and forms the very thick enveloping membrane, the peripodal membrane. The thickened part of the histoblast is the portion which later becomes functional as the developed organ. This portion forms two folds, each fold being composed of several layers of cells. The so-called tracheal veins lie between the two layers of the functional portion of the histoblast. The peripodal membrane and the function portion of the bud are both direct outgrowths of the hypodermis.

There are also other structures which, according to Villanes, are homologous with the histoblasts; these are the so-called optic imaginal buds. (Fig. 43.) The eyeless larva, as is common with the other eyeless Dipterous larvæ, avoids a too strong light, and this perception of light through the integument is probably due to the presence of these optic histoblasts. If a ray of light is concentrated on the region of these optic imaginal buds the response is much quicker than if the light be concentrated on another part of the body. The optic imaginal buds are rectangular in shape and are like other imaginal buds in possessing a peripodal membrane and a wider functional portion of the histoderm, which later develops into the optic ganglion. In the central cavity there are several tracheæ and some loose mesodermal tissue.

X. The Nervous Tissue.

The central nervous system extends along the median line of the ventral surface as a series of ganglia connected by nerve cords. The ganglia are more closely approximated in the anterior portion of the chain and some of the ganglia are united to form the brain. (Fig. 44.) Behind the brain, which comprises the supra-œsophageal ganglion, there is the sub-œsophageal ganglion and a chain of ten ganglia. Just back of the sub-œsophageal ganglion there are four closely approximated ganglia. These are so closely applied that there is no connecting nerve cord. Posterior to these, there are six ventral ganglia which are widely separated and are joined by the slender ventral nerve cord. The ganglia are roughly pyramidal in shape and give off four large nerve trunks; two nerve branches from each side and these soon divide and redivide into finer and finer branches. The terminal, or tenth ganglion (Fig. 45), is larger than the others and gives off four large nerve trunks from the base of the pyramid. The other ganglia also give off prominent nerve trunks; the one rising from the basal apex of the ganglion and going to the muscles of the body wall, while the other, which rises from the middle of the side of the ganglion, goes to the viscera. The muscular branch can easily be seen in looking at the live animal. The first ganglion after the thoracic group lies in the sixth segment, and posterior to this there is one ganglion in each segment.

The brain proper consists of the supra-œsophageal ganglion, which lies above the œsophagus and is connected with the sub-œsophageal ganglion by the œsophageal commissures, so that the brain completely embraces the œsophagus. (Fig. 46.) In front of the brain and extending across it is the frontal ganglion, which composes a part of the sympathetic system. Anterior to this and on either side are the optic imaginal buds. These are small oval swellings of the optic nerve. There are two other nerves given off from the supra-œsophageal ganglion, one going to the antenna, the antennal branch, and the other running to the labium. Going out from the sub-œsophageal are three branches which connect with the mouth parts.

These serve to control the mandible, maxilla, and the labium, and are known as the mandibular, maxillary, and the labial branch.

The brain in its finer structure is exceedingly complex. (Fig. 47.) There are several histological elements, the various cell elements and the fibrillar or Punktsubstanz. The cell elements form the cortical portion of the brain, while the central portion of the brain is composed of minute granules and interlacing fibers. These fibers appear as cut ends in the sections, so that the whole medullary portion appears finely granular. In the cellular portion of the brain there are three distinct kinds of cells. There is first an outer layer of cortical cells which differ somewhat in size, but are always larger than the cells which compose the cell mass lying just within. Then there is the mass of rather smaller and rounded cells which lie on either side of the central body and extend almost around the brain. Among this cell mass may be seen a few very large cells with very darkly staining nuclei. These evidently represent the large motor cells described by Kenyon in his work on the brain of the bee. The same elements may be found in the sub-œsophageal ganglion, although there is not so much definiteness in limiting the cell mass and there are fewer of the large cells. (Fig. 48.) There is here, in common with other Dipterous larvæ, no sign of the complicated mushroom bodies. In the sub-œsophageal ganglion there is a greater amount of the Punktsubstanz in proportion to the size than there is in the supra-œsophageal ganglion.

The thoracic and ventral ganglia show the same histological elements as the brain. (Figs. 49-50.) The arrangement of these elements is in general the same. There is an outer cortex layer of cells, the inner portion is composed of the cell mass with a few large cells interspersed, and a central portion which is composed of the Punktsubstanz. This arrangement is common to both the ventral and thoracic ganglia. Each nerve consists of an axis cylinder, which has a striated appearance in a longitudinal section which is due to the fine fibrillæ, and then the enveloping membrane or neurilemma (Fig. 51).

XI. The Pupa.

When the larva reaches its full size, it has stored up a great amount of reserve food material in the sheets of adipose tissue; it ceases to feed and becomes very sluggish until the last larval skin is shed and the pupa emerges. Pupation here usually takes place some time in April. The pupa is shorter than the larva and is proportionately wider. (Fig. 52.) When it first emerges it is very soft and very transparent, but as the parts becomes chitinized they turn a rich brown. The compound eyes, antennæ, the labial palps and other mouth parts can be seen through the pupal skin, though the head is not marked off from the rest of the body. The three pairs of thoracic legs are short and do not reach beyond the first abdominal segment. The abdominal portion consists of the usual six segments, pure white save for the dark golden band down the ventral and lateral surfaces. On each of the lateral lines there are two brown projections, a large one near the posterior portion of the segment and a smaller one midway. On each median surface there are others; one on each side in the first two abdominal segments and two in the next four. The posterior segment is, of course, sexually modified. The tracheal system of the pupa is closed, as there are no spiracles present.

Since the posterior extremity is changed to conform to the corresponding segments of the adult, the sexual modifications are indicated. The female may at once be recognized by the long triangular valves which are to constitute the ovipositor. The posterior segment of the male shows no such modification, but ends bluntly and shows the transverse anal opening. There are three large lateral spines upon each side of the posterior segment, while the other segments only have one large and one small projection. The spines all project backwards and serve to assist the animal in locomotion by giving it sufficient hold in the soft wood in which the pupa lies. It also enables it to creep to the surface before its final transformation. Such aids are found in very many Dipterous larvæ and Dipterous pupæ, for example in Dicranota, Tipula, and Bibio. If the pupa could not come to the surface before the emergence of the fly much damage would inevitably result and a perfect adult would be rare indeed. If the pupa is extracted from its burrow it very shortly establishes itself in an equaly convenient position just beneath the surface.

XII. The Fly.

The fly emerges toward the end of April or the beginning of May, and is a very handsome fellow with his gay coloring of red, brown, or yellow. (Fig. 53.) The distinctive feature of the crane-flies, according to Comstock, is the presence of the transverse V-shaped suture, and this feature is very marked in this species. The wings are long and narrow, with a characteristic venation, the veins being partially fused at the proximal end. The ovipositor is composed of two long, horny pointed valves, well fitted for depositing the egg in firm substances. The power of flight is not well developed and the ability to walk is also poor. The long legs are so feebly attached to the body that they are easily broken off. This species of Ctenophora ranges from Vancouver Island to California. In conclusion I wish to express my indebtedness to Prof. Trevor Kincaid, of the University of Washington, without whose aid and encouragement this paper would have suffered a great deal.

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